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Tumor-Associated Antigens Detected by Autologous Sera in Urine of Patients with Solid Neoplasms[1]

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Twenty-four-hour urine samples were procured from cancer patients and normal donors. The urine samples were processed and concentrated by centrifugation and ultrafiltration (10,000 MW cutoff). The processed urine samples were tested for the presence or absence of tumor-associated antigens by the complement-fixation assay. Autologous serum was used as the source of antibody. Ninety-two percent (55/60) of cancer patients were positive for the antigens in their urine. Within the cancer patient group, 86% (31/36) sarcoma patients, 100% (17/17) melanoma patients, and 100% (7/7) carcinoma patients were positive. In contrast, only 7% (2/27) normal donors were positive in this assay. Antibody activity of the sera reacting to the urine from cancer patients was removed by absorption with biopsied tumor specimens but not with normal skin or muscle suggesting that the antigens detected in urine of cancer patients were tumor associated.

INTRODUCTION

We reported the detection of tumor-associated antigens in the urines of patients bearing sarcomas and some other malignancies [3, 4, 6]. The antigens could be detected using concentrated, lyophilized, and reconstituted urine as an antigen and autologous or allogeneic sera as antibody sources in the complement fixation test. Antigen excretion was measured at intervals during the patient's clinical course. In sarcoma patients, antigen excretion decreased following surgical resection of the tumor and remained low unless the malignancy recurred.

In this report, we described a new method for preparing urines for testing in the complement fixation test. Our study has been expanded to include a larger number of sarcoma patients and normal donors and patients with melanomas and carcinomas.

MATERIALS AND METHODS

Patients. Sixty patients undergoing treatment by the UCLA Division of Surgical Oncology for a variety of solid neoplasms (sarcomas, melanomas, and carcinomas) and 27 normal donors participated in the study. Age and sex distributions of the participants are listed in Table 1.

Urines. Twenty-four-hour urine samples were collected. During collection the samples were refrigerated, after addition of sodium azide, to prevent bacterial growth. Sample size ranged from 200 ml to 12 liters from cancer patients and 1.0 to 3.0 liters from normal donors. The urines were processed by centrifugation at 17,000*g* for 10 min at 4°C and filtered through Whatman No. 1 filter paper. The filtrates were concentrated to approximately 35 ml by ultrafiltration on an Amicon DC-2 concentrator equipped with a hollow fiber cartridge having a 10,000 MW cutoff. Samples were further concentrated to 1–10 ml by Aquac

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TABLE 1

DIAGNOSES, SEX, AND MEAN AGE OF URINE DONORS

Type of malignancy	Sex	No. of individuals	Age range	Mean age
Sarcomas	M	19	15–67	35.4
	F	17	18–70	39.6
Melanomas	M	13	18–79	52.8
	F	4	23–55	45.3
Carcinomas	M	4	44–92	63.3
	F	3	53–62	57.0
Control (healthy)	M	21	19–48	29.3
	F	6	24–37	31.2

IIA (Calbiochem, La Jolla, Calif.) and dialyzed against 0.025 *M* phosphate-buffered saline, pH 7.2, containing 0.05% sodium azide (PBS). The processed urine was stored at −20°C until testing.

Serum. Multiple serum samples obtained by venipuncture from all participants were decomplemented by incubation at 56°C for 30 min, aliquotted, and stored in liquid nitrogen.

Complement fixation assay. The components of the ultramicrocomplement fixation assay, described previously [3], included 2 μl each of concentrated urine, decomplemented autologous serum, human umbilical cord serum as a complement source, and hemolysin-sensitized sheep red blood cells (0.5% v v). Doubling dilutions of urine were reacted against three dilutions of autologous serum (1:4, 1:8, and 1:16). Antigen activity was determined by the highest dilution of antigen which resulted in at least 50% inhibition of hemolysis using the lowest dilution of serum which was not anticomplementary. Controls for the anticomplementary activities of the urine and serum were included in each testing. Samples were considered antigen-positive only if the titer observed in the presence of serum was two or more dilutions beyond the anticomplementary titer of antigen alone.

Absorption. Serum was absorbed with autologous tumor cells, or skin and muscle obtained from outside the tumor margins. Cell suspensions were prepared under sterile conditions by pressing finely minced tissue through a 60-mesh sieve. The cells were washed with PBS, treated by glycine–HCl buffer, pH 2.7, for 5 min at 4°C to remove bound antibody [5], and then washed again three times with PBS.

Serum was diluted 1:4 with PBS and mixed with equal volumes of packed cells,

TABLE 2

PRESENCE OF URINARY ANTIGENS DETECTABLE BY COMPLEMENT FIXATION USING AUTOLOGOUS SERUM[a]

Group	Positive individuals/ total tested	Percentage positive	Range of titers (reciprocal)	Mean ± SE
Sarcoma	31/36	86	≤2–1024	134 ± 28.1
Melanoma	17/17	100	8–4096	278 ± 177.3
Carcinoma	7/7	100	16–128	56 ± 13.9
Total cancer patients	55/60	92		
Normal controls	2/27	7	≤2–16	2.5 ± 0.5

[a] Concentrated urines were titrated in the presence of three dilutions of autologous serum (1:4, 1:8, and 1:16). The antigen titer was determined as the highest antigen dilution resulting in approximately 50% or less hemolysis using the lowest dilution of serum that was not anticomplementary. A sample was considered positive if the titer of sample + serum was at least two dilutions beyond the anticomplementary activity of the urine alone. An individual was considered positive if a positive antigen titer of greater than 1:8 was observed.

then incubated at 37°C for 1 hr with frequent mixing and at 4°C overnight. After the cells were sedimented at 4500*g* for 5 min, the serum was removed and tested. The absorbed and unabsorbed sera were titrated simultaneously with antigens of autologous urine, urine from another cancer patient, and urine from a normal donor. The unabsorbed serum reacted at different levels to each antigen. One unit of antigen was defined as the highest dilution of that particular antigen which showed reactivity with

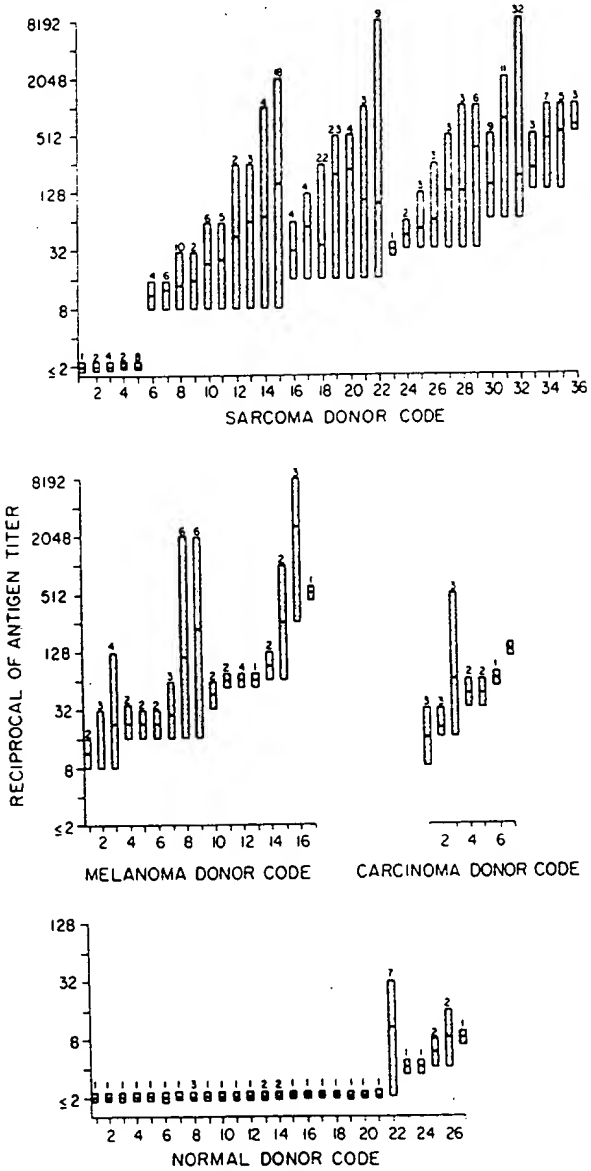

FIG. 1. Range of antigen titers observed for individual participants in the study. Patients are listed by code number with each bar representing the range of observed positive urinary antigen titers for an individual. The dark mark across each bar represents the mean antigen titer for that range. The number above each bar represents the number of samples. The line crossing the figure near a titer of 1:8 represents our cutoff for determining if a patient is positive for urinary antigen. If any sample shows a positive titer above 1:8, the patient is considered positive.